

Activity pattern of the Neotropical harvestman *Neosadocus maximus* (Opiliones, Gonyleptidae): sexual and temporal variations

Francini Osses and Taís M. Nazareth: Programa de Pós-graduação em Ecologia e Conservação de Recursos Naturais, Universidade Federal de Uberlândia, CP 593, 38400-902 Uberlândia, MG, Brazil

Glauco Machado¹: Departamento de Ecologia, Instituto de Biociências, Rua do Matão, Travessa 14, no 321, 05508-900, São Paulo, SP, Brazil

Abstract. We investigated the activity pattern of males and females of the neotropical harvestman *Neosadocus maximus* Giltay 1928 focusing on behavioral variations between day and night and also between summer and winter. Our study also proposes a new method for quantifying arthropod behavior in captivity based on totally random samplings, which minimizes problems of pseudoreplication, so common in studies of behavioral repertoires. Eighteen individuals (nine males and nine females) collected at the Parque Estadual Intervales, São Paulo state, Brazil, were maintained in the laboratory from June 2003 to February 2004 for qualitative and quantitative observations. Thirty-four behavioral acts grouped in five behavioral categories were recorded, with “resting” (53.1%) and “exploration” (30.8%) being the most frequent. The behavioral repertoire of males (32 acts) was more diversified than that of females (29 acts). Moreover, there was a significant effect of sex (male \times female) on the frequency of the behavioral categories: females expended more time resting than did males, whereas males expended more time in exploration and grooming activities than did females. There was also a significant effect of time of day (day \times night) and of season (winter \times summer) on the frequency of the behavioral categories: individuals were most active at night and during summer. Finally, temperature, but not humidity, had a positive effect on the activity of the individuals. In conclusion, the activity of *N. maximus* has sexual, daily, and seasonal variations, which are both quantitative and qualitative.

Keywords: Behavioral sampling, behavioral repertoire, ethogram, rhythm, seasonality, Zeitgeber

Arachnids are generally considered to be primarily nocturnal, although there are exceptions among representatives of the orders Araneae, Solifugae, and Opiliones (Cloudsley-Thompson 1978; Foelix 1996; Punzo 1998; Hoenen & Gnaspini 1999). However, the activity patterns of few arachnids have been quantitatively addressed so that studies on spiders roughly divide the species into day- or night-active (e.g., Marc 1990; Alderweireldt 1994). Additionally, quantitative data on sexual and temporal variations in the activity patterns of arachnids have rarely been reported. Among scorpions, for instance, the seasonal surface activity of mature males differs markedly from that of females in many species (Warburg & Polis 1990). At least for some North American scorpions, females are more active in spring and fall, which is coincident with the peak of insect abundance, whereas males are more active on the surface during midsummer, corresponding to the period of greatest abundance of virgin females (Polis & Farley 1979a, b; Polis 1980).

Most harvestmen species seem to concentrate their activity at night, when temperature decreases and relative air humidity increases (Todd 1949). Certain species, however, are more active during daytime, and others may be found active throughout the day (Bishop 1950; Pfeifer 1956; Williams 1962; Hoenen & Gnaspini 1999). The literature on rhythms is scarce for harvestmen, but includes some information on sexual differences in the activity patterns. Gnaspini (1996), for instance, described that ovigerous females of the Brazilian cavernicolous harvestman *Goniosoma spelaum* Mello-Leitão 1922 (Gonyleptidae, Goniosomatinae) leave the cave to forage more frequently than males. Recently, the behavioral reper-

tory of males and females has been compared quantitatively in two gonyleptid harvestmen from Brazil, namely *Discocyrtus oliverioi* Soares 1945 (Pachylinae) and *Mischonyx cuspidatus* Roewer 1913 (Gonyleptinae). In both species females fed more frequently than males, whereas males were involved in social interactions more frequently than females (Elpino-Campos et al. 2001; Pereira et al. 2004). Although these two studies describe the daily activity pattern of the harvestmen, they do not provide data on seasonal variation in the frequency of the behavioral categories because both were done only during the summer (December to February).

The great majority of the information regarding seasonal variations in harvestmen is related to the phenology of the species (e.g., Phillipson 1959; Friebe & Adis 1983; Martens 1993; Tsurusaki 2003). These phenological studies provide data on the occurrence of different instars throughout the year, but generally do not deal with seasonal differences in behavior. However, examples of seasonal changes in behavior may be found in the neotropical harvestmen *Acutisoma longipes* Roewer 1913 and *Goniosoma albiscriptum* Mello-Leitão 1932 (Gonyleptidae, Goniosomatinae), which form aggregations inside caves only during the dry and cold season (Machado et al. 2000; Willemart & Gnaspini 2004a) and reproduce only during the wet and warm season (Machado & Oliveira 1998; Willemart & Gnaspini 2004b). The most comprehensive study on seasonal changes in harvestman behavior has been conducted by Gnaspini et al. (2003) with *G. spelaum*. The authors showed that exits of individuals from the cave were related to sunset time, occurring earlier in winter (June/July) than in summer (December/January). However, the advancement in the time during which individuals leave the cave in summer does not exactly follow

¹ Corresponding author. E-mail: glaucom@ib.usp.br

the advancement in the time of sunset in comparison to winter data, suggesting a change in the phase angle.

Ethograms are important starting points for ethological research and for understanding the biology and ecology of a wide range of animals (Lehner 1996). This methodology allows qualitative and quantitative comparisons between the behavioral repertoires of different species, as well as individuals of the same species belonging to different sexes. In this study we used such an approach to compare the behavioral repertoire of males and females of the neotropical harvestman *Neosadocus maximus* Giltay 1928 (Gonyleptidae, Gonyleptinae), a species commonly found in forest fragments of the Atlantic Forest in southeastern Brazil. Additionally, we investigated for the first time whether the activity pattern of males and females varies between day and night, and also between summer and winter. Our study also proposes a new method for quantifying arthropod behavior in captivity based on totally random samplings, which minimizes problems of pseudoreplication, so common in studies of behavioral repertoires.

METHODS

Taxonomic note.—The genus *Neosadocus* is defined by a cluster of tubercles on the dorso-basal portion of femur IV of males (A.B. Kury pers. comm.), and includes species separated by subtle variations (Kury 2003). Misidentifications are commonplace in the literature and the species treated in this study as *Neosadocus maximus* has been previously named *N. variabilis* by Gnaspini (1996), Machado & Pizo (2000), and Willemart (2002) or *Neosadocus* sp. (Willemart et al. 2007). Two other studies (Willemart & Gnaspini 2003; Castanho & Pinto-da-Rocha 2005), however, properly identified the species as *N. maximus*. The species studied by Machado & Vital (2001) and identified as *Neosadocus* aff. *variabilis* is probably closely related to *N. maximus*, but it is a distinct taxon. Voucher specimens are deposited in the Museu de Zoologia da Universidade de São Paulo (MZSP, Brazil).

Collection and rearing.—Individuals of *N. maximus* were collected in a dense Atlantic Forest fragment at the Parque Estadual Intervales (24°14'S; 48°04'W; 800 m elev.), close to the municipality of Ribeirão Grande, southern São Paulo state, Brazil. The annual rainfall in the region ranges from 2,000 to 3,000 mm and the mean annual temperature ranges from 12° C to 20° C. The climate is seasonal with two well-defined seasons. The dry and cold season (hereafter called winter) lasts from April to September, has a mean monthly rainfall of 139 mm, mean monthly air temperature of 17.4° C, and mean monthly air humidity of 79% (data taken *in loco* during 5 yr, 1998–2002). The rainy and warm season (hereafter called summer) lasts from October to March, has a mean monthly rainfall of 306 mm, mean monthly air temperature of 20.9° C, and mean monthly air humidity of 83% (data taken *in loco* during 5 yr, 1998–2002).

Behavioral observations were done in the laboratory based on 18 specimens: nine females and nine males. Each individual received a number from 1 to 18 marked on their dorsal scute with synthetic enamel paint for model airplanes (cf. Elpino-Campos et al. 2001). Individuals were maintained in a communal terrarium (40 × 90 cm base, 20 cm height) containing soil, two individuals of an aroid plant, and pieces

of rotting logs where they could rest and find protection from light. They were fed pieces of dead cockroaches three times a week and always received additional food before the behavioral samplings. From April to September the conditions in the laboratory were (mean ± SD): temperature of 22.1 ± 2.0° C, humidity of 75.6 ± 7.1%, and photoperiod of 12L:12D. Between October and March we changed the laboratory conditions in order to simulate natural climatic oscillations in nature: temperature was 25.5 ± 1.2° C, humidity was 82.0 ± 5.4%, and photoperiod was 13L:11D. Although temperature variation in the laboratory was not the same as in the field, the magnitude of the difference between winter and summer was nearly the same, and the air humidity was close to that measured in the study site. Moreover, the laboratory photoperiod, which is the most important Zeitgeber for many organisms (including arthropods, see Aschoff 1960), reproduced the same seasonal variation observed in nature from winter to summer. Thus, we believe that the results obtained in captivity can be generalized to harvestmen under field conditions.

Behavioral sampling.—The ethogram of *N. maximus* was derived from preliminary 18 h of qualitative *ad libitum* observations of captive individuals (Table 1). The behavioral acts recorded during this phase were classified into five major groups of activities or “behavioral categories”: feeding, exploration, self-grooming, resting, and social interactions (Table 2). Quantitative behavioral observations were done in two distinct seasons: winter samples ($n = 24$) were gathered from June to August 2003, and summer samples ($n = 24$) were gathered from December 2003 to February 2004. In this study we used a new sampling procedure that was designed to minimize the problem of pseudoreplication (see Discussion). According to our protocol, each sample consisted of 1 h of observation randomly selected from midnight to 23:00. After a given 1 h period was selected it was excluded from the next assortments. No sample was done with less than 24 h of interval from the previous sample. During each sample, the behavioral acts were recorded at 1 min intervals (“fixed-interval time point sampling” sensu Martin & Bateson 1993). Only one individual was recorded at each 1 min interval, and its identity was based on a list of random numbers ranging from 1 to 18, in which each number corresponded to the mark of one individual. At the end of each sample, 60 behavioral acts were always recorded (one per minute), and each individual was scanned from one to nine times (median of three times, $n = 48$ sections). When a selected individual was not visible in the terrarium, we searched for it by gently raising the pieces of rotting logs. A flashlight with red filter was used for the nocturnal observations (cf. Elpino-Campos et al. 2001).

Statistical analyses.—The data were classified into two periods: winter and summer. Within each of these two categories, they were also classified in day and night samples. Since the photoperiod differed between the two seasons, the number of day samples was 12 in winter and 13 in summer. Finally, the data were classified into males ($n = 9$) and females ($n = 9$). A log-linear analysis was used to test for the effects of the period of the day, season, and sex on the frequency of the behavioral categories. This analysis is based on fitting a hierarchical log-linear model to the cell frequencies so that it is possible to restate the problem of analyzing multi-way

Table 1.—Behavioral acts of the harvestman *Neosadocus maximus* based on 18 h of ad libitum observations of nine males and nine females maintained under laboratory conditions. Behavioral acts marked with an asterisk were modified from Elpino-Campo et al. (2001).

Behavioral acts	Description
Feeding*	To manipulate organic matter using the chelicerae and pedipalps, inserting it into the mouth. This behavior may be accomplished with the body standing on the legs or with the venter in contact with the substrate. When two or more individuals manipulate the same piece of food at the same time, this behavior is classified as "sharing food."
Walkabout carrying food	To walk carrying food with the chelicerae and pedipalps.
Drinking water	To put the mouthparts in direct contact with the water.
Walkabout – legs II up*	To walk with legs II extended sideways or towards the front without touching the substrate.
Walkabout – legs II touching the substrate	To walk with legs II extended sideways or towards the front touching the substrate.
Motionless – legs II down	To stay motionless with legs II extended sideways downward without touching the substrate.
Motionless – legs II up	To stay motionless with legs II extended sideways upward (Fig. 2).
Motionless – legs II back	To stay motionless with legs II extended back and upward.
Motionless – legs II touching the substrate*	To stay motionless with legs II touching the substrate.
Cleaning the pedipalps*	To pass the tip of the pedipalps through the chelicerae and mouthparts.
Leg threading*	Process of cleaning in which each leg is passed through the chelicerae and mouthparts until the end of tarsus is reached.
Cleaning the venter, dorsum, legs III or IV using legs II	Process of indirect cleaning in which legs II are passed on some parts of the body (venter, dorsum, legs III or IV) and are then cleaned, passing them through the chelicerae and mouthparts until the end of tarsus is reached.
Resting*	To remain motionless with legs retracted over the body and with the venter in contact with the substrate (Fig. 1). While resting individuals may be: (1) isolated, at least 3 cm from each other or (2) in groups, at least three individuals at 0–2 cm apart from each other with legs overlapping. Individuals may rest in protected (under rotting logs) or unprotected sites (open areas of the terrarium).
Aggressive posture	To remain motionless with the pedipalps raised toward another individual.
Mutual leg tapping	Occurs when two individuals walk around each other touching themselves with legs I and II. This is a preliminary step of an intra-sexual fight accomplished exclusively by males.
Intertwining legs IV	After mutual leg tapping, the individuals turn their backs to each other and intertwine legs IV. In this position, each male tries to capsize each other promoting a sudden upward movement in which a male brings femur IV close to the body and pinches legs IV of the opponent.
Chasing other individual	Occurs when a male (generally the winner of the fight) runs after his opponent.
Fighting for food*	Any aggression toward another individual possessing food. It involves attack with pedipalps and touching with the first two pairs of legs.
Attacking with the pedipalps	To approach another individual not possessing food and aggressively attack it with the pedipalps.
Touching other individuals*	Physical contact between two individuals established using legs I and/or II, without aggressive posture (see above) by either individual.

frequency tables in terms that are very similar to ANOVA. According to this model, the natural logarithm of the expected cell frequency is written as an additive (linear) function of effects, including first-order effects and higher-order interactions among the categorical variables (Christensen 1997). This statistical procedure is more than just an alternative form of the chi-square test, which is sensitive to the most common violation of their assumptions, i.e., lack of independence between observations (Kramer & Schmidhammer 1992). Its strength lies in that it can be extended to quite complicated contingency tables involving several variables.

A multiple regression was used to test the relationship between temperature and humidity (independent variables) and number of behavioral acts accomplished per hour (dependent variable). Regarding this analysis, it is important to stress the following: (a) there is no significant correlation between the independent variables ($r = 0.139$; $P = 0.346$), so that there is no problem of multicollinearity in the multiple regression; (b) the number of behavioral acts does not include the category "resting" since we were interested in testing the influence of temperature and humidity only on the activity of the individuals; (c) data from both summer and winter were

used, comprising 48 samples. A two-factor ANOVA was performed to test for differences in the number of behavioral acts per hour (excluding "resting") between day and night in both seasons.

RESULTS

General patterns.—The behavioral repertory of captive individuals of *Neosadocus maximus* comprises 34 behavioral acts (Table 1). No behavioral act directly related to reproduction, such as courtship, copulation, egg-laying, or parental care has been recorded in captivity. During the 48 h of quantitative observations, four acts of the behavioral repertory were not recorded, all belonging to the category "social interactions" (numbers 27–29 and 31 in Table 2). The most common behavioral categories were "resting" and "exploring," which accounted for 53.1% and 30.8% of the total of observations, respectively (Table 2). Most of the "resting" observations were of individuals grouped underneath rotting logs (Fig. 1), whereas most of the "exploring" observations were of motionless individuals with their second pair of sensorial legs upwards (Table 2; Fig. 2). The great majority of the individuals fed alone, and food sharing was recorded in

Table 2.—Frequency of each behavioral act for captive adults of the harvestman *Neosadocus maximus* in two seasons (winter and summer). In parentheses is the total number of behavioral acts observed for the individuals in each season.

Behavioral acts	Frequency of the behavioral acts (%)					
	Males (n = 9)		Females (n = 9)		Total (n = 18)	
	Winter (697)	Summer (661)	Winter (743)	Summer (779)	Winter (1440)	Summer (1440)
FEEDING	3.73	14.54	3.23	13.99	3.48	14.23
1. With the body erect	3.01	11.5	2.15	9.50	2.57	10.42
2. With the body in contact with the substrate	0.29	1.83	0.82	2.43	0.56	2.15
3. Walkabout carrying the food	0.14	0.61	0.13	1.16	0.14	0.90
4. Food sharing	0	0.15	0	0.51	0	0.35
5. Drinking water	0.29	0.45	0.13	0.39	0.21	0.41
EXPLORATION	34.85	39.94	21.27	28.50	27.85	33.75
6. Walkabout – legs II up	4.3	3.78	2.16	2.18	3.19	2.92
7. Walkabout – legs II touching the substrate	5.16	4.69	4.98	3.34	5.07	3.96
8. Motionless – legs II down	2.73	11.04	2.69	7.32	2.71	9.03
9. Motionless – legs II up	14.20	12.56	7.27	11.04	10.63	11.73
10. Motionless – legs II back	5.45	5.60	1.48	2.95	3.40	4.17
11. Motionless – legs II touching the substrate	3.01	2.27	2.69	1.67	2.85	1.94
SELF-GROOMING	5.90	7.10	3.22	5.26	4.51	6.12
12. Cleaning the pedipalps	2.44	3.93	1.48	2.95	1.94	3.40
13. Leg threading – legs I	1.00	1.66	0.54	1.28	0.76	1.46
14. Leg threading – legs II	1.59	0.45	0.94	0.77	1.25	0.63
15. Leg threading – legs III	0.29	0.61	0.13	0.26	0.21	0.42
16. Cleaning legs III with legs II	0	0.15	0	0	0	0.07
17. Leg threading – legs IV	0.44	0.15	0	0	0.21	0.07
18. Cleaning legs IV with legs II	0.14	0	0	0	0.07	0
19. Cleaning the venter with legs II	0	0.15	0	0	0	0.07
20. Cleaning the dorsum with legs II	0	0	0.13	0	0.07	0
RESTING	53.37	36.91	69.86	50.45	61.87	44.22
21. Resting alone protected	10.90	3.63	8.34	10.53	9.58	7.35
22. Resting alone unprotected	5.16	6.20	3.77	0.90	4.44	3.33
23. Resting in group protected	37.02	27.08	57.07	38.76	47.36	33.40
24. Resting in group unprotected	0.29	0	0.68	0.26	0.49	0.14
SOCIAL INTERACTIONS	2.15	1.51	2.42	1.80	2.29	1.68
25. Aggressive posture	0	0	0	0.13	0	0.07
26. Mutual leg tapping	0.14	0	-	-	0.07	0
27. Intertwining legs IV	0	0	-	-	0	0
28. Chasing another individual	0	0	-	-	0	0
29. Male vs. female fight	0	0	0	0	0	0
30. Fighting for food	0	0.45	0.13	0.13	0.07	0.28
31. Attacking with the pedipalps	0	0	0	0	0	0
32. Male touching male	1.29	1.06	-	-	0.69	0.49
33. Female touching female	-	-	0.81	0.77	0.35	0.42
34. Female touching male, and vice versa	0.72	0	1.48	0.77	1.11	0.42

only 0.3% of the observations (Table 2). Probably as a means to avoid food sharing or even food plundering, some individuals carried the food away before starting consumption. Grooming behavior occurred when the harvestmen were moving around the terrarium and, more frequently, after feeding (when both legs and pedipalps were cleaned) and social interactions, such as fights and mutual leg tapping.

Sexual variation.—The behavioral repertoires of males and females of *N. maximus* differed both qualitatively and quantitatively. The behavioral acts number 26–29 were accomplished exclusively by males and are related to a ritual of intra-sexual aggression (Table 1). Five fights between males were observed in the qualitative observations, but during the

quantitative samplings this behavior was not recorded. A fight starts with two males walking around each other, touching the opponent with the first and the second pairs of legs. After about three to five complete turns the males stop and, if the fight escalates, they turn their backs to each other and intertwine their fourth pairs of heavily-armed legs. In this position, each male moves the femur IV pinching the opponent's leg between the apophysis and spines of their own femur and coxa IV. The fight ends when one of the contenders leaves the place.

The log-linear analysis showed that there was a significant effect of sex (male vs female) on the frequency of the behavioral categories (Table 3). Females were seen resting and interacting with conspecifics more frequently than males



Figures 1, 2.—Adult *Neosadocus maximus*. 1. Female of *N. maximus* resting under a rotting log in captivity. Note that the legs are retracted over the body and the venter is in contact with the substrate. 2. Male of *N. maximus* walking on the vegetation in the field. Note the second pair of sensory legs extended sideways and touching the substrate (photos: B.A. Buzatto).

(Fig. 3). Males, on the other hand, were more active, exploring and grooming more than females (Fig. 3). Individuals of both sexes fed with a similar frequency (Fig. 3). Aggressiveness among males, however, may explain why females rested

Table 3.—Results of the log-linear analysis used to test for the effects of time (day vs night), season (summer vs winter), and sex (male vs female) on the frequency of behavioral categories accomplished by captive individuals of the harvestman *Neosadocus maximus*.

Model	Chi-square	df	P
Season × Time × Sex	1.587	4	0.811
Season × Time	8.175	8	0.417
Time × Sex	0.803	4	0.938
Season × Sex	8.498	12	0.745
Season (summer vs winter)	160.960	8	< 0.001
Time (night vs day)	33.929	8	< 0.001
Sex (male vs female)	76.722	8	< 0.001

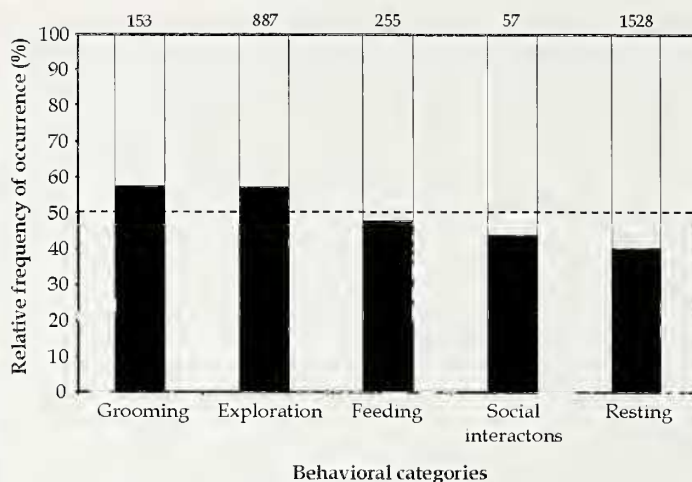


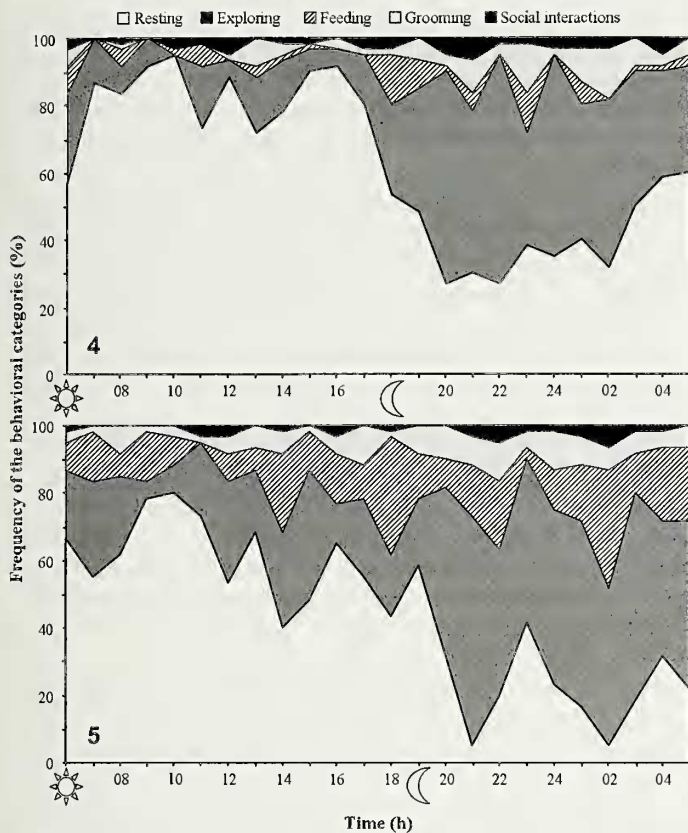
Figure 3.—Comparison of the relative frequency of occurrence of the behavioral categories observed for males (black bars) and females (white bars) of the harvestman *Neosadocus maximus*. The numbers above the bars indicate the total number of records in each behavioral category.

aggregated more frequently than males, both in winter and summer (Table 2).

Temporal variations.—The log-linear analysis showed that there was a significant effect of the period of time (day vs night) on the frequency of the behavioral categories (Table 3). Individuals of *N. maximus* remained inactive during most of the daylight hours, when they were resting underneath rotting logs (Figs. 4, 5). In winter, individuals became active at about 17:00 h, i.e., one hour before dusk (Fig. 4). In summer, activity began at about 19:00 h, just after dusk (Fig. 5). Night activities included exploring the environment by walking around the terrarium and feeding on pieces of dead cockroaches (Figs. 4, 5). Male-male fights were also restricted to the nocturnal period. In winter, activities started to decrease at about 02:00 h, whereas in summer activities started to decrease only at about 05:00 h, when most of the individuals were seen resting or moving to the shelters underneath rotting logs (Figs. 4, 5).

The log-linear analysis also showed that there was an effect of season (winter vs summer) on the frequency of the behavioral categories (Table 3). Even though resting and exploring were the most common activities in both seasons, there was an increase in the frequency of resting from summer to winter, and a decrease in the frequency of exploration from summer to winter (Table 2). Additionally, there was a marked reduction in the frequency of feeding from one season to the other: during winter, in nearly 4% of the observations individuals were feeding versus 14% during summer (Table 2). The frequency of the categories self-grooming and social interactions were similar between seasons (Table 2). It is worthy to note that the interactions between sex and season as well as time and season have no significant effect on the frequency of the behavioral categories (Table 3).

A positive relationship between the number of behavioral acts and the temperature was detected, but this relationship was not significant for humidity (Table 4). The mean number of behavioral acts accomplished per hour during the night was greater than during the day in both seasons ($F_{(1, 44)} = 99.915$;



Figures 4, 5.—Daily activity schedule of the harvestman *Neosadocus maximus* in (4) winter and (5) summer. The moon and the sun indicate dusk and dawn, respectively.

$P < 0.001$; Fig. 6). Additionally, when comparing seasons, the mean number of behavioral acts accomplished per hour was greater in summer than in winter ($F_{(1, 44)} = 25.368$; $P < 0.001$; Fig. 6).

DISCUSSION

Comparisons with other species.—Harvestmen are excellent model organisms for behavioral studies because individuals of many species are abundant in the field and are easily maintained in captivity, where they behave in a similar way to that observed in the field (e.g., Capocasale & Bruno-Trezza 1964; Elpino-Campos et al. 2001; Willemart 2001; Pereira et al. 2004), facilitating quantitative and qualitative comparisons between species. In this study, we used the neotropical harvestman *Neosadocus maximus* in order to investigate sexual and temporal variations in the behavior of captive individuals. The data obtained here may also be compared with those previously obtained for other harvestman species reared under similar conditions. The number of behavioral acts recorded for

Table 4.—Multiple regression between the number of behavioral acts (excluding resting) accomplished by captive individuals of the harvestman *Neosadocus maximus* and two independent variables: temperature and humidity ($F_{(2, 45)} = 4.80$; $P = 0.013$).

Independent variable	b	$F_{(1, 44)}$	P
Humidity	0.066	1.174	0.284
Temperature	0.485	7.425	0.009

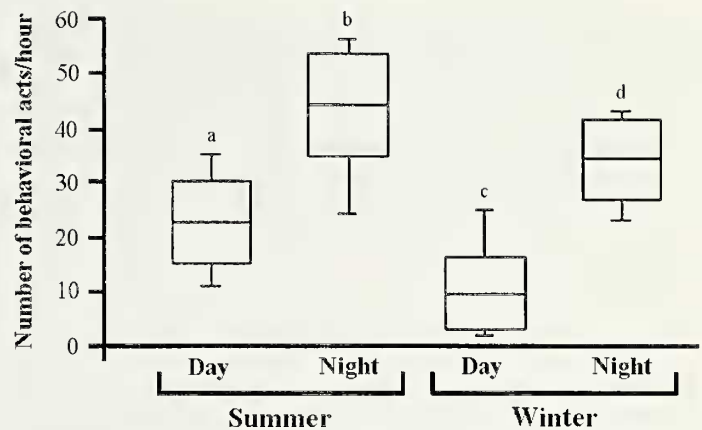


Figure 6.—Comparison of the number of behavioral acts (excluding resting) accomplished per hour by individuals of the harvestman *Neosadocus maximus* during day and night in two seasons (summer and winter). The horizontal lines represent the mean, the boxes represent the standard deviation, and the vertical lines represent the range (minimum and maximum values in each sample). Different letters above the box-plots indicate significant difference with $P < 0.05$.

N. maximus is greater than those recorded for the harvestmen *Discocyrtus oliverioi* (25 acts, Elpino-Campos et al. 2001) and *Mischonyx cuspidatus* (20 acts, Pereira et al. 2004). This difference may be attributed to two main factors: (1) some behavioral acts described in the previous studies were split into two or more different acts in our study (e.g., acts 8–11); (2) *N. maximus*, in fact, presents a number of distinctive behavioral acts that were not recorded before, such as those related to self-grooming (number 16 and 18–20) and social interactions (number 26–28). There was also a marked difference among the three harvestman species in the frequency of the behavioral categories exploration and resting. In *D. oliverioi* and *M. cuspidatus*, exploration was the most common behavioral category, comprising nearly 70% of the observations, whereas in *N. maximus* this category accounted for 28% in winter and 34% in summer. Resting, which ranged from 11 to 17% in *D. oliverioi* and *M. cuspidatus*, was the most frequent behavioral category for *N. maximus* comprising 44 to 62% of the observations. These discrepancies could be related to differences in the sampling methods, since Elpino-Campos et al. (2001) and Pereira et al. (2004) concentrated the quantitative observations at night (when the individuals were more active), whereas in the present study we scattered the behavioral samplings equally throughout the whole day. In fact, if we analyze the data obtained only in the night samples for *N. maximus*, the frequency of resting drops to 22% and of exploration increases to 52%. Therefore, we believe that the data presented in this study are a more realistic scenario of time allocation in harvestmen during the whole day.

Contrary to *D. oliverioi* and *M. cuspidatus*, individuals of *N. maximus* did not copulate or lay eggs in captivity, so that the behavioral category reproduction, which comprised one to seven behavioral acts and accounted for 0.1 to 1.8% of the observations in the previous studies, was not included here. Field observations indicate that *N. maximus* oviposits on the undersurface of leaves and eggs are attended by females (G. Machado unpubl. data). It is possible that the lack of

appropriate oviposition sites in the terrarium have constrained the reproductive activity of the females. Males, on the other hand, accomplished certain behavioral acts, such as intrasexual aggressions, that are possibly related to territorial defense and reproduction. Male-male fights have already been described for several harvestman species (e.g., Pabst 1953; Parisot 1962; Edgar 1971; Mora 1990; Macías-Ordóñez 2000; Willemart et al. 2006), but this is the first record of this kind of agonistic behavior among gonyleptids. The use of legs IV, which bear large spines and tubercles, shed light on the behavioral roles of the leg armature in *N. maximus* and other gonyleptid harvestmen as well. Until now, the only function attributed to the armature of legs IV in males was to deliver a nipping upon manipulation, which has been interpreted as a defensive behavior against potential predators (Bristowe 1925; Capocasale & Bruno-Trezza 1964; Gnaspini & Cavalheiro 1998; Machado 2002). A more detailed description of the fights and the functional morphology of legs IV in *N. maximus* will be described elsewhere (Willemart et al. unpubl. data).

Sexual and temporal variation.—Males and females of *N. maximus* differed in the relative frequency of the behavioral categories, with males exploring more frequently than females. This result contrasts with those obtained for *D. oliverioi* and *M. cuspidatus*, in which females fed and explored more frequently than males (Elpino-Campos et al. 2001; Pereira et al. 2004). Since both *D. oliverioi* and *M. cuspidatus* reproduced in captivity, the higher frequency of feeding activities in females compared to males was attributed to the accumulation of energy for egg production and maturation (Pereira et al. 2004). The higher frequency of exploration by males compared to females in *N. maximus* may be explained by at least two non-exclusive factors: (1) in this species males seem to be territorial (G. Machado unpubl. data) and thus need to invest time patrolling and exploring their territories; (2) females did not oviposit in captivity and, thus, were not continuously producing eggs, which would reduce the demand for food resources and consequently decrease the frequency of activities related to foraging. Since self-grooming occurs more frequently when individuals are moving around and after feeding (Pereira et al. 2004), the fact that males explored more than females may account for the higher frequency of grooming compared to females.

The daily activity of the individuals of *N. maximus* in captivity was predominantly nocturnal, and light seems to be the most important Zeitgeber promoting synchronization of the activity rhythm. This observation is congruent with data previously obtained in the field, where 100% of the feeding observations in this species occurred at night (Machado & Pizo 2001). The activity pattern, however, presents seasonal variations with a clear change in the phase angle between activity and sunset/sunrise hours from winter to summer. During winter, individuals left the shelters earlier, one hour before dusk. The peak of activity occurred nearly at 20:00 h, and at 02:00 h there was a marked decrease in the frequency of behavioral acts not related to resting. In contrast, during summer, individuals left the shelter only after dusk, remained active throughout the night, and returned to the shelter at about 05:00 h, nearly one hour before the onset of light. In some aspects, this pattern of activity is similar to that described for the cavernicolous harvestman *Goniosoma*

spelaeum, in which the individuals also left the cave earlier in winter when compared to summer (Gnaspini et al. 2003). However, contrary to the present study, individuals of *G. spelaeum* returned later to the shelter during winter. The authors attributed this change in the phase angle to the time available to forage outside the cave, which is shorter during summer. It is possible that captive individuals of *N. maximus* can find food faster than individuals of *G. spelaeum* in the wild and thus can return earlier to their shelters. Another possibility is that different species respond differently to seasonal variations in biotic and abiotic factors. We suggest that future studies investigate how hunger and other physiological constraints modulate long-term changes in the biological rhythms of harvestmen.

In our study we demonstrate that temperature, but not humidity, has a positive relationship with the activity of captive individuals of *N. maximus*, which may explain why the individuals were more active during summer. A similar result has been reported by Capocasale & Bruno-Trezza (1964), who reared individuals of the gonyleptid *Acanthopachylus aculeatus* (Kirby 1818) in the laboratory and showed that foraging activity seems to be directly related to temperature. However, the predominant nocturnal activity of the species in the field and in the laboratory should not be attributed to temperature since the latter decreases at night. We believe that the decrease in light intensity at dusk, rather than the decrease in temperature, controls the beginning of activity in *N. maximus*. After darkness, however, air temperature can be an important abiotic factor determining harvestmen activity; the warmer the night the more active are the individuals. Additionally, it is important to stress the role of phylogeny in determining the activity pattern of harvestmen. *N. maximus* belongs to a clade in which most species are mainly nocturnal. Predominant diurnal activity among gonyleptids seems to be restricted to the clade composed of the subfamilies Progonyleptoidellinae + Caelopyginae (Hoenen & Gnaspini 1999).

Methodological approach.—The density of harvestmen used in our terrarium (18 individuals in 0.36 m²) is certainly much higher than the density in the wild, which is no more than 0.04 individuals/m² (G. Machado unpub. data). Crowding may increase the frequency of some behavioral acts, mainly those grouped in the behavioral category “social interactions”. However, for the great majority of the behavioral acts (including those grouped in the behavioral categories “feeding,” “exploration,” “self-grooming,” and “resting”) the density of individuals in captivity probably had no evident effect. Moreover, since these last four behavioral categories comprise nearly 98% of the behavioral acts in both seasons, we believe that the results obtained under captive conditions for *N. maximus* provide a realistic scenario of time allocation throughout the day and also in different seasons. The lack of appropriate oviposition sites in our terrarium probably inhibited some behavioral acts, mainly those related to reproduction. We acknowledge this drawback of our laboratory work and recommend that future studies try to reproduce as good as possible the oviposition sites of the study species in the rearing terrarium.

Despite some minor problems mentioned above, harvestmen are very convenient animals to keep in captivity since many species are relatively easy to maintain and may live for several months or even years (Willemart 2007). In our case, to

study animals in the laboratory provided the opportunity to quantify the behavior of a great number of individuals in each sampling section and also made it possible to compare the behavioral schedule of the very same subjects in two seasons, which would be very difficult in the wild. Simulating natural variations in climatic conditions, we showed that there were differences in the frequency of five behavioral categories recorded for *N. maximus*, both at the scale of the day and of the year (winter vs summer). This temporal variation may be endogenously regulated and/or dependent on the environmental variables we manipulated, but we can not actually differentiate between these two possibilities.

In this study we also proposed a new sampling procedure for quantitative ethograms that minimizes problems of pseudoreplication. Traditionally, behavioral samplings in quantitative ethograms are accomplished at regular intervals and all subjects are recorded at each scan. Using this procedure, one will face at least two situations that violate the assumption of independent observations required by many statistical tests, especially the frequently used chi-square (see discussion in Kramer & Schmidhammer 1992): (1) one behavioral act may influence the chance of another behavioral act being accomplished in sequence; (2) if the individual A is interacting with the individual B, this behavioral act will be counted twice because the interaction is reciprocal. Yet the "traditional" ethograms have an advantage: the amount of information in each sampling section is high; more specifically $n \times s$, where n is the number of individuals and s is the number of scans. Using the method proposed here, the amount of data in each sampling section is only s because only one individual is scanned at a time. Sampling one individual per scan, however, is exactly the solution for the situation (2) above. Additionally, the median number of times the same individual was scanned per sampling section along the entire period of our study was three. These repeated samplings on the same individuals were spaced out in time, which attenuates the problem exposed in the situation (1). Finally, we also took care of spreading the sampling sections along the time, spacing them with intervals of at least 24 h in order to attenuate the possible influence the activities accomplished in one day could have on activities accomplished in the following day. This is not a standard procedure in "traditional" ethograms, which concentrate the samplings in the periods of more activity or in fixed times of the day.

We are aware that sampling the same individuals (in our case 18 harvestmen) is, per se, a source of pseudoreplication, but changing each animal for another one after it was sampled does not seem a reasonable procedure in behavioral studies and would demand a huge quantity of animals. Anyone interested in controlling potential differences among individuals may include each one of them as additional factors in the log-linear analysis. Here we avoided this approach because our main goal was to detect general patterns.

Conclusions.—In conclusion, we demonstrated that the activity pattern of the neotropical harvestman *N. maximus*, determined here by the frequency of five behavioral categories, shows sexual and temporal variations. These variations are both quantitative and qualitative since some behavioral acts are restricted to one sex or period of the day. The sampling protocol proposed here should be used in future studies

dealing with behavioral repertoires and ethograms because it minimizes the problems of pseudoreplication and provides a more realistic view of the allocation of time and energy for different activities. The great advantage of this method is that it provides a suitable sampling design that generates independent data, instead of trying to correct the problem of non-independence a posteriori using complicated statistical procedures. Our protocol should be useful as a standard method in behavioral samplings not only for harvestman, but for the study of any arthropod reared in captivity.

ACKNOWLEDGMENTS

We are grateful to Gustavo S. Requena and Bruno A. Buzatto for collecting some individuals used in this study, to Adriano B. Kury for helping with the identification of the studied species, to Paulo De Marco Jr. and Rafael Lourenço for helping with the statistical analyses, and to Pedro Gnaspini, Sonia Hoenen, Rodrigo H. Willemart, Bruno A. Buzatto, Paulo Enrique C. Peixoto, Gail Stratton, and two anonymous reviewers for insightful comments on an early draft of the manuscript. The authors are supported by grants from CAPES (FO and TMN) and Fundação de Amparo à Pesquisa do Estado de São Paulo (GM 02/00381-0).

LITERATURE CITED

- Alderweireldt, M. 1994. Day/night activity rhythms of spiders occurring in crop-rotated fields. *European Journal of Soil Biology* 30:55–61.
- Aschoff, J. 1960. Exogenous and endogenous components in circadian rhythms. Pp. 11–28. *In* *Biological Clocks*. Cold Spring Harbor Symposia on Quantitative Biology, Volume 25. Long Island Biological Association, New York.
- Bishop, S.C. 1950. The life of a harvestman. *Nature Magazine* 43:264–267, 276.
- Bristowe, W.S. 1925. Notes on the habits of insects and spiders in Brazil. *Transactions of the Royal Entomological Society of London* 1924:475–504.
- Capocasale, R. & L. Bruno-Trezza. 1964. Biología de *Acanthopachylus aculeatus* (Kirby, 1819), (Opiliones; Pachylinae). *Revista de la Sociedad Uruguaya de Entomología* 6:9–32.
- Castanho, L.M. & R. Pinto-da-Rocha. 2005. Harvestmen (Opiliones: Gonyleptidae) predated on treefrogs (Anura: Hylidae). *Revista Ibérica de Aracnología* 11:43–45.
- Cloudsley-Thompson, J.L. 1978. Biological clocks in Arachnida. *Bulletin of the British Arachnological Society* 4:184–191.
- Christensen, R. 1997. *Log-Linear Models and Logistic Regression*. Second edition. Springer-Verlag Inc. New York, New York. 508 pp.
- Edgar, A.L. 1971. Studies on the biology and ecology of Michigan Phalangida (Opiliones). *Miscellaneous Publications, Museum of Zoology, University of Michigan, Ann Arbor* 144:1–64.
- Elpino-Campos, A., W. Pereira, K. Del-Claro & G. Machado. 2001. Behavioural repertory and notes on natural history of the neotropical harvestman *Discocyrtus oliverioi* (Opiliones: Gonyleptidae). *Bulletin of the British Arachnological Society* 12:144–150.
- Foelix, R.F. 1996. *Biology of Spiders*. Second edition. Oxford University Press, New York. 330 pp.
- Friebe, B. & J. Adis. 1983. Entwicklungzyklen von Opiliones (Arachnida) im Schwarzwasser-Überschwemmungswald (Igapó) des Rio Tarumã Mirim (Zentralamazonien, Brasilien). *Amazoniana* 8:101–110.
- Gnaspini, P. 1996. Population ecology of *Goniosoma spelaeum*, a cavernicolous harvestman from southeastern Brazil (Arachnida: Opiliones: Gonyleptidae). *Journal of Zoology* 28:137–151.

- Gnaspini, P. & A.J. Cavalheiro. 1998. Chemical and behavioral defenses of a Neotropical cavernicolous harvestman *Goniosoma spelaeum* (Opiliones, Laniatores, Gonyleptidae). *Journal of Arachnology* 26:81–90.
- Gnaspini, P., F.H. Santos & S. Hoenen. 2003. The occurrence of different phase angles between contrasting seasons in the activity patterns of the cave harvestman *Goniosoma spelaeum* (Arachnida, Opiliones). *Biological Rhythm Research* 34:31–49.
- Hoenen, S. & P. Gnaspini. 1999. Activity rhythms and behavioral characterization of two epigeal and one cavernicolous harvestmen (Arachnida, Opiliones, Gonyleptidae). *Journal of Arachnology* 27:159–164.
- Kramer, M. & J. Schmidhammer. 1992. The chi-squared statistic in ethology: use and misuse. *Animal Behaviour* 44:833–841.
- Kury, A.B. 2003. Annotated catalogue of the Laniatores of the New World (Arachnida, Opiliones). *Revista Ibérica de Aracnología*, vol. especial monográfico 1:1–337.
- Lehner, P.N. 1996. *Handbook of Ethological Methods*. Garland STPM Press, New York. 672 pp.
- Machado, G. 2002. Maternal care, defensive behavior, and sociality in Neotropical *Goniosoma* harvestmen (Arachnida, Opiliones). *Insectes Sociaux* 49:1–6.
- Machado, G. & P.S. Oliveira. 1998. Reproductive biology of the Neotropical harvestman *Goniosoma longipes* (Arachnida, Opiliones: Gonyleptidae): mating and oviposition behaviour, brood mortality, and parental care. *Journal of Zoology* 246:359–367.
- Machado, G. & M.A. Pizo. 2000. The use of fruits by the Neotropical harvestman *Neosadocus variabilis* (Opiliones, Laniatores, Gonyleptidae). *Journal of Arachnology* 28:357–360.
- Machado, G., R.L.G. Raimundo & P.S. Oliveira. 2000. Daily activity schedule, gregariousness, and defensive behavior in the Neotropical harvestman *Goniosoma longipes* (Arachnida: Opiliones: Gonyleptidae). *Journal of Natural History* 34:587–596.
- Machado, G. & D.M. Vital. 2001. On the occurrence of epizoic algae and liverworts on the harvestmen *Neosadocus* aff. *variabilis* (Opiliones: Gonyleptidae). *Biotropica* 33:535–538.
- Macías-Ordóñez, R. 2000. Touchy harvestmen. *Natural History* 109:58–61.
- Marc, P. 1990. Nycthemeral activity rhythm of adult *Clubiona corticalis* (Walckenaer, 1802) (Araneae, Clubionidae). *Acta Zoologica Fennica* 190:279–285.
- Martens, J. 1993. Bodenlebende Arthropoda im zentralen Himalaya: Bestandsaufnahme, Wege zur Vielfalt und Ökologische Nischen. Pp. 23–250. *In* *Neue Forschungen im Himalaya*. (U. Scheinfurth, ed.). *Erdkundliches Wissen*. Volume 112. Franz Steiner Verlag, Stuttgart.
- Martin, P. & P. Bateson. 1993. *Measuring Behaviour: an Introductory Guide*. Second edition. Cambridge University Press, New York. 222 pp.
- Mora, G. 1990. Parental care in a Neotropical harvestman, *Zygopachylus albouarguinis* (Arachnida, Opiliones: Gonyleptidae). *Animal Behaviour* 39:582–593.
- Pabst, W. 1953. Zur Biologie der mitteleuropäischen Trogliden. *Zoologische Jahrbücher, Abteilung für Systematik, Ökologie und Geographie der Tiere* 82:1–156.
- Parisot, C. 1962. Étude de quelques opilions de Lorraine. *Vie et Milieu* 13:179–197.
- Pereira, W., A. Elpino-Campos, K. Del-Claro & G. Machado. 2004. Behavioral repertory of the Neotropical harvestman *Ilhaia cuspidata* (Opiliones, Gonyleptidae). *Journal of Arachnology* 32:22–30.
- Pfeifer, H. 1956. Zur Ökologie und larvalsystematik der Weberknechte. *Mitteilungen aus dem Zoologischen Museum in Berlin* 32:59–104.
- Phillipson, J. 1959. The seasonal occurrence, life histories and fecundity of harvest-spiders (Phalangida, Arachnida) in neighborhood of Durham City. *Entomologist's Monthly Magazine* 95:134–138.
- Polis, G.A. 1980. Seasonal patterns and age-specific variation in the surface activity of a population of desert scorpions in relation to environmental factors. *Journal of Animal Ecology* 49:1–18.
- Polis, G.A. & R.D. Farley. 1979a. Behavior and ecology of mating in the cannibalistic scorpion, *Paruroctonus mesaensis* Stahnke (Scorpionida: Vaejovidae). *Journal of Arachnology* 7:3–46.
- Polis, G.A. & R.D. Farley. 1979b. Characteristics and environmental determinants of natality, growth and maturity in a natural population of the desert scorpion *Paruroctonus mesaensis* (Scorpionida: Vaejovidae). *Journal of Zoology* 187:517–542.
- Punzo, F. 1998. *The Biology of Camel Spiders (Arachnida, Solifugae)*. Kluwer Academic Publishers, Dordrecht, The Netherlands. 301 pp.
- Todd, V. 1949. The habits and ecology of the British harvestmen (Arachnida, Opiliones), with special reference to those of the Oxford District. *Journal of Animal Ecology* 18:209–229.
- Tsurusaki, N. 2003. Phenology and biology of harvestmen in and near Sapporo, Hokkaido, Japan, with some taxonomical notes on *Nelima suzukii* n.sp. and allies (Arachnida: Opiliones). *Acta Arachnologica* 52:5–24.
- Warburg, M.R. & G.A. Polis. 1990. Behavioral responses, rhythms, and activity patterns. Pp. 224–246. *In* *The Biology of Scorpions*. (G.A. Polis, ed.). Stanford University Press, Stanford, California.
- Willemart, R.H. 2001. Egg covering behavior of the neotropical harvestman *Prouitobates ornatus* (Opiliones, Gonyleptidae). *Journal of Arachnology* 28:249–252.
- Willemart, R.H. 2002. Cases of intra- and inter-specific food competition among Brazilian harvestmen in captivity (Opiliones, Laniatores, Gonyleptidae). *Revue Arachnologique* 14:49–58.
- Willemart, R.H. 2007. Rearing and maintenance of harvestmen in captivity. Pp. 520–524. *In* *Harvestmen: The Biology of Opiliones*. (R. Pinto-da-Rocha, G. Machado & G. Giribet, eds.). Harvard University Press, Cambridge, Massachusetts.
- Willemart, R.H., M.C. Chelini, R. de Andrade & P. Gnaspini. 2007. An ethological approach to a SEM survey on sensory structures and tegumental gland openings of two neotropical harvestmen (Arachnida, Opiliones, Gonyleptidae). *Italian Journal of Zoology* 74:39–54.
- Willemart, R.H., J.P. Farine, A.V. Peretti & P. Gnaspini. 2006. Behavioral roles of the sexually dimorphic structures in the male harvestman *Phalangiun opilio* (Opiliones, Phalangiidae). *Canadian Journal of Zoology* 84:1763–1774.
- Willemart, R.H. & P. Gnaspini. 2003. Comparative density of hair sensilla on the legs of cavernicolous and epigeal harvestmen (Arachnida: Opiliones). *Zoologischer Anzeiger* 242:353–366.
- Willemart, R.H. & P. Gnaspini. 2004a. Spatial distribution, displacement, gregariousness and defensive behavior in the Brazilian cave harvestman *Goniosoma albiscriptum* (Arachnida, Opiliones, Laniatores). *Animal Biology* 54:221–235.
- Willemart, R.H. & P. Gnaspini. 2004b. Breeding biology of the cavernicolous harvestman *Goniosoma albiscriptum* (Arachnida, Opiliones, Laniatores): sites of oviposition, egg-batches, characteristics and subsocial behaviour. *Invertebrate Reproduction and Development* 45:15–28.
- Williams, G.C. 1962. Seasonal and diurnal activity of harvestmen (Phalangida) and spiders (Araneida) in contrasted habitats. *Journal of Animal Ecology* 31:23–42.